RECEIVED
CENTRAL FAX CENTER

SEP 3 0 2004

Attorney Docket No. P66378US0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Franz Josef MEYER-ALMES

Via facsimile
703-872-9306

Application No.: 09/762,304 Art Unit: 1642

Filed: April 16, 2001 Examiner: Dr. M. YU

For: CHEMOSENSITIVITY MEASUREMENT USING CASPASE ACTIVITY

TRANSMITTAL

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith by facsimile are:

☒ Response and Request for Examiner Initialed Form PTO 1449, including attachment

☒ Petition for extension of time

☒ Fee payment ☒ Payment Form PTO-2038 (credit card) for $110 is attached.

☐ Charge $ * to Deposit Account No. 06-1358.

☐ Small entity status established in connection with the subject application.

Fee Calculation					
Excess Claims					
	№ of Claims	Highest № Paid For	Excess Claims	Small Entity Fee	Large Entity Fee
Total	*	⊖ * =	0	⊗ $9 = $	⊗ $18 = $
Ind.	*	⊖ * =	0	⊗ $43 = $	⊗ $86 = $
[ ] Multiple Dependent Claims (1st Filing)				⊕ $145 =	⊕ $290 = $
Extension of Time Fee				$	$110
Total Fee Due				$	$110

☒ If a petition for extension of time is necessary, but not enclosed, then this acts as the petition. Charge any fees additionally necessary in connection herewith to Deposit Account No. 06-1358.

JACOBSON HOLMAN PLLC
400 Seventh Street, N. W.
Washington, D.C. 20004-2201
Tel. (202) 638-6666
Fax (202) 393-5350
Date: September 30, 2004

By [signature]
William E. Player
Registration No. 31,409

R:\Home\rthomas\2004\SEPTEMBER\P66378US0 trans.wpd

SEP 3 0 2004

Attorney Docket No. P66378US0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Franz Josef MEYER-ALMES

Via facsimile 703-872-9306

Application No.: 09/762,304 Art Unit: 1642

Filed: April 16, 2001 Examiner: Dr. M. YU

For: CHEMOSENSITIVITY MEASUREMENT USING CASPASE ACTIVITY

RESPONSE AND REQUEST FOR EXAMINER INITIALED FORM PTO 1449

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The instant paper, transmitted by facsimile, responds to the Office Action mailed December 30, 2003, following Notice of Appeal filed June 30, 2004.

Claims 27-41, previously presented, are pending.

Claims 27-41 stand rejected under (1) 35 USC 112, ¶1, for allegedly containing *new matter*, and under (2) 35 USC 112, ¶1, for allegedly lacking enablement. In an apparently separate rejection, i.e., based on subject matter unique to the claim at issue, claim 38 is rejected under 35 USC 112, ¶1, for allegedly containing *new matter*. Reconsideration of the aforesaid §112 rejections of record is requested.

With respect to the *new-matter* rejection, under §112, ¶1, of claim 38, the subject matter at issue does not constitute *new matter*, allegations to the contrary in the statement of rejection, notwithstanding. Support for the language in claim 38 that allegedly constitutes new matter can be found in the specification (page 7, lines 120), i.e. (emphasis in original):

> It is essential that the <u>accumulated</u> caspase activity is measured. In this approach, cells, e.g., tumor cells of a patient, are incubated with at least one substance (e.g., cytostatic agent) for an extended period of time so that all effective pharmaceuticals, even the slowly acting, have had enough time to induce apoptosis. Subsequently, for example, a substrate present in lysis buffer is added, and after the cell lysis has been effected, the total caspase activity formed, i.e., the accumulated activity, of the cellular suspension is measured.

Accordingly, withdrawal of the rejection appears to be in order.

With respect to the §112, ¶1, rejections applied against claims 27-41, collectively, first of all, Applicant disagrees with the allegation, set forth in the statement of rejection, that the written description and enablement requirements of §112, ¶1, are satisfied for only *coumarin-based* fluorogenic substrates containing DEVD. Indeed, it appears that only *coumarin-based* fluorogenic substrates containing DEVD are known in the art, according to the statement of rejection. Contrary to the allegations found in the statement of rejection, fluorogenic, DEVD-containing substrates besides those based on coumarin are known, which are useful in accordance with the present claims.

The use of non-coumarin-based fluorogenic, DEVD-containing substrates in caspase activity assays is disclosed, e.g., in *Bioorganic & Medical Chemistry Letters, 9*, 3231-3236, 1999 (Liu) (copy submitted herewith). Liu teaches the use of coumarin-based substrates, primarily, to determine caspase-3 activity (Liu, bottom of page 3231). However, besides coumarin-based substrates, Liu discloses the use of rhodamine110-based (fluorogenic) substrates in caspase-activity assays. Moreover, the rhodamine110-based (fluorogenic) substrates exhibit higher turnover rates and sensitivity than exhibited coumarin-based substrates (Liu, Figure 1).

The §112, ¶1, rejections rely on the allegation that *only* coumarin-based fluorogenic, DEVD-containing substrates are known and, so, useful in accordance with the presently claimed invention. The allegation is incorrect, as explained above. Therefore, withdrawal of the rejections for alleged failure to satisfy the written description and enablement requirements of §112, ¶1, appears to be in order.

Both the *new-matter* rejection under §112, ¶1, and the rejection alleging lack of enablement under §112, ¶1, as applied against claims 27-41, collectively, are based on the same reasoning. Both rejections rely on allegations that the *generic scope* of the claims is not supported by the subject application as originally filed. As explained below, the rejections cannot be sustained because they are legally erroneous, i.e., the rejections fail to apply the correct legal standards for determining satisfaction of the written description and enablement requirements of §112, ¶1.

MPEP 706.03(a)(1) sets forth the minimum requirements that must be satisfied in order to reject claims under §112, ¶1, i.e. (*emphasis added*):

> A *prima facie* showing must contain the following elements: . . .
>
> ii) *support* for factual findings relied upon in reaching this conclusion . . .
>
> Office personnel must provide documentary evidence (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) as the form of *support* used in establishing the factual basis of a *prima facie* showing .

Little, if any, of the "support" needed to sustain the rejections, i.e., by way of "documentary evidence (e.g., scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents, " is provided in the outstanding Office Action.

Nothing by way of scientific reasoning or evidence is provided in the outstanding Office action to rebut applicant's assertion of usefulness for the generic scope of the present claims. In order to sustain a rejection for lack of enablement under §112, first paragraph, and shift the burden to a patent applicant, the PTO must cite evidence in support of any allegations of non-compliance with §112, ¶1, in addition to explaining *why* it doubts the truth of statements made in the specification. *In re Sichert*, 196 USPQ 209 (CCPA 1977). Even in an unpredictable area, such as chemistry, the PTO must advance reasons why a patent applicant's broad assertion of usefulness is not true. *In re Bowen*, 181 USPQ 48 (CCPA 1974). The PTO has the initial burden to make a *prima facie* showing inconsistent with the *presumption* that the requirements of §112, ¶1, are satisfied. *Sichert, supra*.

Failure to satisfy the requirements of §112, ¶1, is not established by mere allegations of undue breadth, i.e., that claims read on non-disclosed embodiments. *Horton v. Stevens*, 7 USPQ2d 1245 (BPA&I 1988). In order to satisfy the requirements of §112, first paragraph, "it is not necessary to embrace in the claims or describe in the specification all possible forms in which the claimed principle may be reduced to practice." *Smith v. Snow*, 294 U.S. 1, 11 (1935). The law does not require an applicant to describe in his specification every conceivable embodiment of the invention. *SRI Int'l v. Matsushita Elec. Corp. of America*, 227 USPQ 577, 586 (Fed. Cir. 1985).

"The test for sufficiency of support . . . is whether the disclosure of the application 'reasonably conveys to the artisan that the inventor had possession at the time of the later claimed subject matter.'" *Vas-Cath Inc. V. Mahukar*, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). *In re Kaslow*,

217 USPQ 1089, 1096 (Fed. Cir. 1983). Presence or absence of literal support is not the issue. *Ex parte Harvey*, 3 USPQ2d 1626 (Bd. Pat. App. & Inter. 1987). To comply with the written description requirement the specification need not describe the claimed invention *in ipsis verbis*." *In re Edwards*, 196 USPQ 465 (CCPA 1978). The proper test is whether the disclosure reasonably conveys to the skilled artisan that the inventor had possession of the claimed subject matter. *Id.* The record fails to show that Applicant did not have possession of the invention as presently claimed

Moreover, allegations to the contrary in the statement of rejection, notwithstanding, failure to satisfy the requirements of §112, ¶1, is not demonstrated merely because the claim scope might, theoretically, cover embodiments that do not work – the function of the claims being not to specifically exclude possibly inoperative embodiments. *Atlas Powder v. E.I. du Pont de Nemours Co.*, 224 USPQ 409 (Fed. Cir. 1984).

Claims 27-41 were rejected under 35 USC 102(b) for allegedly lacking novelty based on Martins, of record. Reconsideration is requested.

For anticipation under § 102 to exist, each and every claim limitation, as arranged in the claim, must be found in a single prior art reference. *Jamesbury Corp. v. Litton Industrial Products, Inc.*, 225 USPQ 253 (Fed. Cir. 1985). The absence from a prior art reference of a single claim limitation negates anticipation. *Kolster Speedsteel A B v. Crucible Inc.*, 230 USPQ 81 (Fed. Cir. 1986). A reference that discloses "substantially the same invention" is not an anticipation. *Jamesbury Corp.* To anticipate the claim, each claim limitation must "*identically* appear" in the reference disclosure. *Gechter v. Davidson*, 43 USPQ2d 1030, 1032 (Fed. Cir. 1997) (*emphasis*

added). To be novelty defeating, a reference must put the public in possession of the identical invention claimed. *In re Donahue*, 226 USPQ 619 (Fed. Cir. 1985).

At least one limitation on the present claims (recited in independent claim 27) is absent from Martins, i.e., the limitation to

> measuring the accumulated caspase activity in the sample without previously separating off the cells.

To induce apoptosis, Martins discloses two alternatives. In alternative 1, cells were treated with 17 µmol/l etoposide for one hour, sedimented at 150 g for 10 minutes, and resuspended in one of two drug-free media. In alternative 2, etoposide was added to a final concentration of 69 µmol/l for continuous exposure experiments. Thereafter, irrespective of which alternative had been chosen for the induction of apoptosis, cytosol and nuclei were prepared. At the start of this cell fractionation process, the cells were washed twice in a serum-free medium, i.e., the cells were separated from their original medium.

Cells undergoing apoptosis show characteristic biochemical and morphological features. These characteristics include the partition of cytoplasm and nucleus into membrane bound vesicles, so-called apoptotic bodies. Such apoptotic bodies undergo "secondary necrosis" which is associated with swelling and partial lysis of the vesicles. Conducting an apoptose assay in the way described by Martins bears the risk that cells have already started to under concomitant release of caspase activity in the medium before the cells are separated from their original activity in the medium before the cells are separated from their original medium by the washing step. Consequently, any caspase

activity released into the medium as a consequence of the presence of a test substance, is not detected because of the separation step taught by Martins.

Further, in contrast to the allegation set forth in the statement of rejection, there is a difference between the accumulated caspase activity assay of the present claims – as embodied in example 1 of the subject application (page 12) – and the art of record and the method described in the art of record.

Example 1 of the subject application refers to application Figure 1 and describes the following:

-- Determination of caspase activity according to the conventional prior art method: 100 µl of Jurkat cells (2.5 x 106/ml) was incubated in the presence of actinomycin D at 5% CO_2 and 37 °C in RPMI medium with 10% FCS and Pen/Strep. After the incubation, the cells were centrifuged by a conventional method and washed with PBS. The pellet was resuspended in 200 µl of lysis buffer, incubated in ice for 10 min. and frozen at -20 °C.

– Determination of caspase activity according to the presently claimed invention: The accumulated caspase activity was determined by mixing 100 µl of the cells incubated with actinomycin D, directly in suspension, with 100 µl of 2x lysis buffer. The lysate was also frozen at -20 °C.

Consequently, in contrast to the conventional prior art methods, the presently claimed invention *excludes* separating off the cells. This *exclusionary* feature of the present claims being

absent from Martins negates anticipation over the reference. *Kolster Speedsteel A B, supra.* Withdrawal of the rejection appears to be in order.

Request for Examiner Initialed Form PTO 1449

On October 16, 2003, an Information Disclosure Statement, including completed form PTO 1449 and a copy of the cited reference, was filed in the PTO. To date, the submitted PTO form 1449, initialed by the Examiner to show consideration of the reference cited thereon, was neither attached to the Office Action nor included with any paper previously issued by the PTO.

Accordingly, it is requested that the PTO return the submitted form PTO 1449, initialed by the Examiner to show that the reference cited, thereon, was considered by the Examiner during prosecution of the subject application.

Favorable action is requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By [signature]
William E. Player
Reg. No. 31,409

400 Seventh Street, NW
The Jenifer Building
Washington, D.C. 20004
Tel. (202) 638-6666
Fax (202) 393-5350
Date: September 30, 2004
WEP/bap
Attachment: *Bioorganic & Medical Chemistry Letters, 9*, 3231-3236, 1999.

R:\Home\rthomas\2004\SEPTEMBER\P66378US0 amd.wpd

Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 3231–3236

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

FLUORESCENT MOLECULAR PROBES V: A SENSITIVE CASPASE-3 SUBSTRATE FOR FLUOROMETRIC ASSAYS

Jixiang Liu, Mahesh Bhalgat, Cailan Zhang, Zhenjun Diwu*, Brian Hoyland, and Dieter H. Klaubert

Molecular Probes, Inc., 4849 Pitchford Avenue, Eugene, OR 97402, U.S.A.

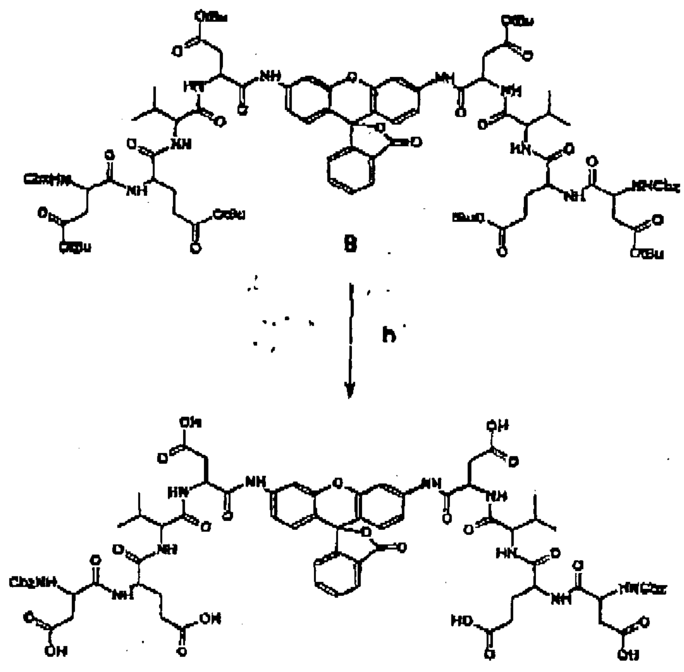
Received 3 September 1999; accepted 11 October 1999

Abstract: (Z-Asp-Glu-Val-Asp)$_2$-Rhodamine 110 [(Z-DEVD)$_2$-Rh 110] was prepared and characterized as a sensitive fluorogenic substrate for the determination of caspase-3 activity.

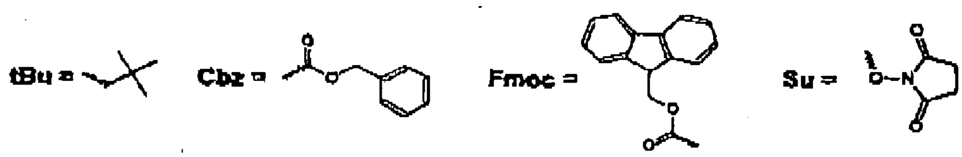
Programmed cell death, the so-called apoptosis, has recently received intensive attention. It is a normal physiological process that occurs during embryonic development as well as in maintenance of tissue homeostasis. However, improperly regulated apoptosis can contribute to several pathological conditions including cancer, Alzheimer's disease, spinal muscular atrophy, ischemic cardiac damage, and autoimmune syndromes.[1,2] One of the earliest and most consistently observed features of apoptosis is the induction of a series of cytosolic proteases, the caspases, which cleave protein substrates and lead to apoptotic morphology.[1-6] Caspase-3, also called appopain or CPP32, has been identified to be a key member of this caspase family of proteases.[7] It has been suggested that activation of the ICE-family proteases and caspase-3 activity are required for several phenotypes associated with apoptosis in mammalian cells.[3,4]

Currently two coumarin-based fluorogenic substrates, Ac-DEVD-AFC and Z-DEVD-AMC, are predominantly used to determine caspase-3 activity.[1-6] However, the low extinction coefficients and short excitation and emission wavelengths of the enzymatic products released from the two coumarin substrates seriously limit the assay sensitivity. Additionally, the residual fluorescence of the two existing substrates and poor wavelength separation of their enzymatic products from the cellular autofluorescence also tend to give high background in microscopic assays.

PII: S0960-894X(99)00566-1

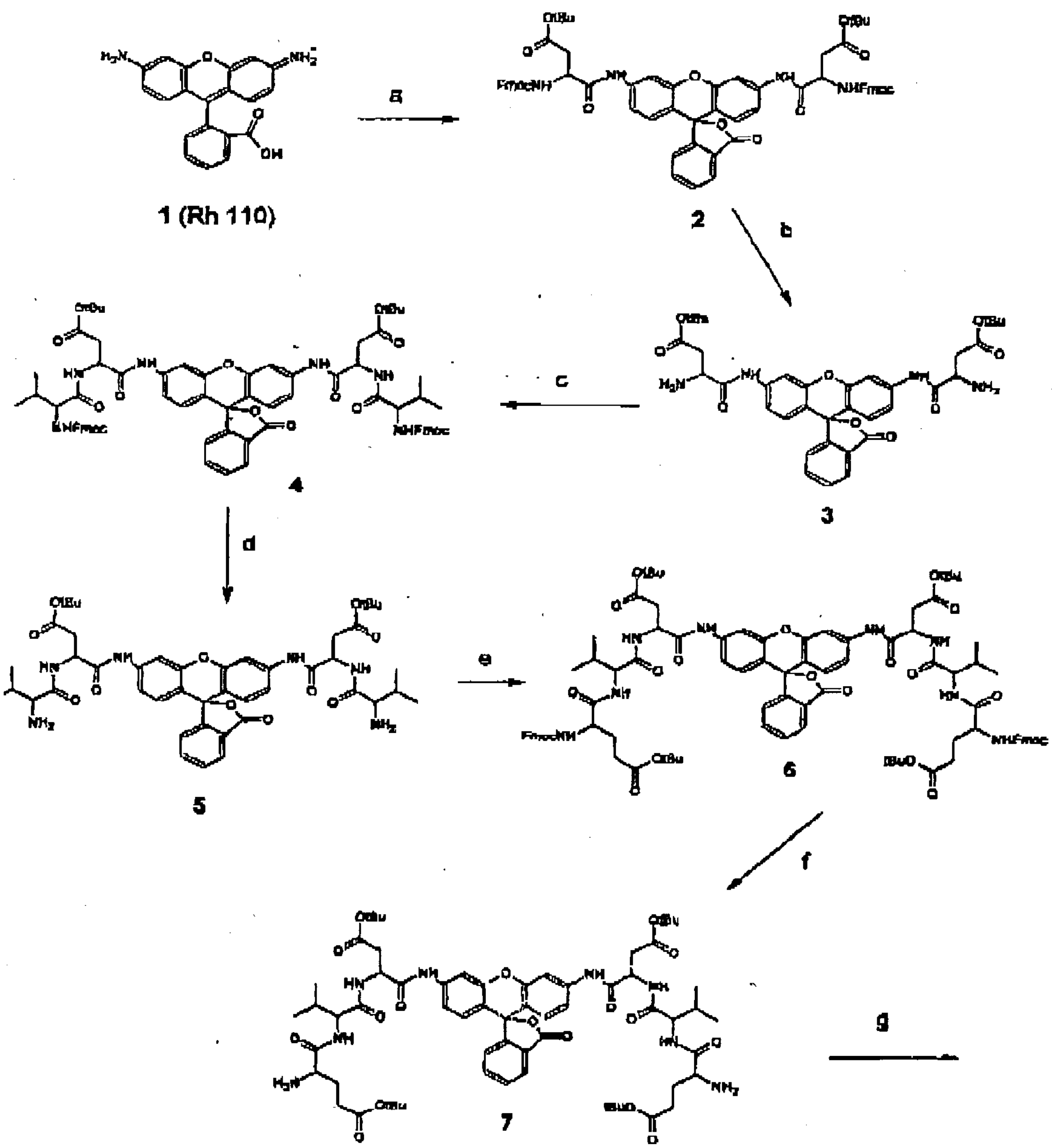


9 $(Z\text{-}DEVD)_2$-Rh 110



Scheme 1. Synthesis of $(Z\text{-}DEVD)_2$-Rh 110.

(a). N-Fmoc-Asp(OtBu)/1-(3-dimethylamino)propyl-3-ethylcarbodiimide hydrochloride (EDC)/pyridine/DMF, yield = 86%; (b). piperidine/chloroform, yield = 83%; (c). N-Fmoc-Val/EDC/pyridine/DMF, yield = 81%; (d). piperidine/chloroform, yield = 92%; (e). N-Fmoc-Glu(OtBu)/EDC/pyridine/DMF, yield = 83%; (f). piperidine/chloroform, yield = 92%; (g). N-Cbz-Asp(OSu)/chloroform, yield = 80%; (h). trifluoroacetic acid/anisole/chloroform, yield = 45%.


1 (Rh 110)
a
2
b
3
c
4
d
5
e
6
f
7
g

$(Z\text{-}DEVD)_2$-Rh 110 (rhodamine 110) was designed to overcome the limitations of the existing coumarin-based caspase substrates, as mentioned above. The substrate was readily prepared as shown in Scheme 1.[5] Initially we prepared tetrapeptide Asp-Val-Glu-Asp, and attempted to couple the peptide with Rh 110 in a single step to give the desired substrates. However, we had difficulties in the coupling of the tetrapeptide with Rh 110 although a variety of conditions were tried. Even coupling of the dipeptide Asp-Val with Rh 110 was a low-yielding reaction. In our hands, it appeared that sequential coupling is the most effective method to prepare the substrate that has been commercialized recently. In general, we found that Rh 110 is much less reactive with bulky acids (such as peptides) compared with smaller acids (such as single amino acids).

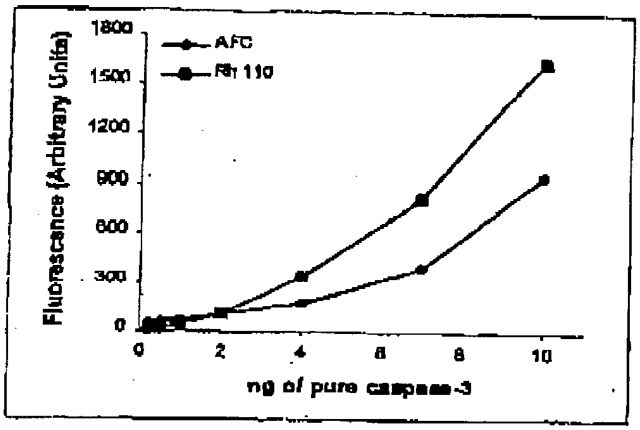


Figure 1. Detection limit of caspase-3 using different DEVD substrates. For the AFC substrate, 44 µL of 25X reaction buffer (250 mM PIPES, pH 7.4, 50 mM EDTA, 2.5% CHAPS, 125 mM DTT) was diluted with 934 µL water. To the reaction solution was added 22.4 µL of a 4.9 mM Z-DEVD-AFC stock solution; for the Rh 110 substrate, 1.79 µL of 12.3 mM $(Z\text{-}DEVD)_2$-Rh 110 stock solution was added to the reaction solution. To the AFC and Rh 110 substrate solutions, various dilutions of the enzyme were added, and incubated at room temperature. The fluorescence data were recorded on a Perkin-Elmer HTS-7000 plate reader with settings: for the AFC assay, Ex = 360 nm, Em = 535 nm, Gain = 40; For the Rh 110 assay, Ex = 485 nm, Em = 535 nm, Gain = 35.

$(Z\text{-}DEVD)_2$-Rh 110 was compared with Z-DEVD-AFC, a fluorogenic caspase-3 substrate that is currently used in a variety of caspase-3 assays. As shown in Figs. 1 and 2, $(Z\text{-}DEVD)_2$-Rh 110 exhibits much higher turnover rate and sensitivity than the AFC substrate. Under our assay conditions, $(Z\text{-}DEVD)_2$-Rh 110 is at least 10-fold more sensitive than Z-DEVD-AFC. It can detect less than 1 ng caspase-3/mL.[4] However, the enzymatic hydrolysis of $(Z\text{-}DEVD)_2$-Rh 110 is a two-step process while the hydrolysis of Z-DEVD-AFC is a single step reaction. The intermediate product, Z-DEVD-Rh 110, is less fluorescent than the final product (Rh

110). The two-step hydrolysis limits the linear dynamic range of $(Z\text{-}DEVD)_2$-Rh 110 substrate as seen in Figure 1. We are in the process of developing a new Rh 110-based substrate that contains only one protease-hydrolyzable amide group.

Table 1. Spectral properties of Z-DEVD-AFC, $(Z\text{-}DEVD)_2$-Rh 110 and their enzymatic products[a]

	λ_{abs} (nm)	$\varepsilon\times10^{-2}$ ($cm^{-1}M^{-1}$)	λ_{F} (nm)	Φ_F
Z-DEVD-AFC	336	139	440	0.44
AFC	376	182	499	0.54
$(Z\text{-}DEVD)_2$-Rh 110	234	551	N/A	Not detected
Rh 110	499	912	519	0.92

[a]The fluorescence quantum yields were determined in PBS buffer (pH 7.2) as described in reference [7]. For the AFC compounds, quinine sulfate was used a reference standard while fluorescein was used as a reference standard for Rh 110.

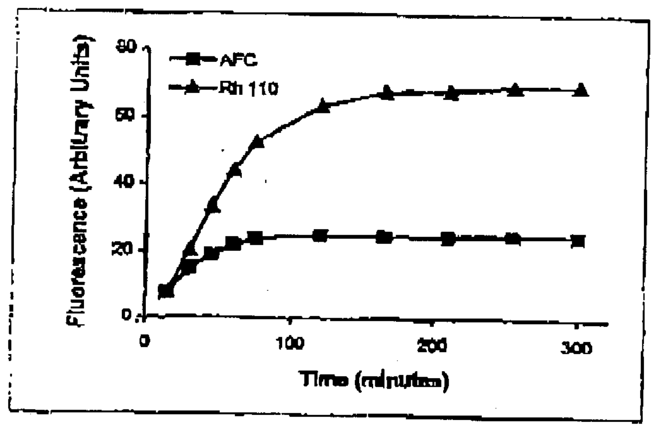


Figure 2. Turnover of $(Z\text{-}DEVD)_2$-Rh 110 (6.25 μM) and Z-DEVD-AFC (25 μM). The enzymatic reactions were run as described in Figure 1, and followed by measuring the fluorescence changes in a CytoFluor II microplate reader using the settings in Figure 1.

As shown in Table 1, Rh 110, the caspase-cleaved product of $(Z\text{-}DEVD)_2$-Rh 110, has maximum absorption at 499 nm that matches very well with 488 nm line of the argon-ion laser. The argon-ion laser is the most popular excitation light source for flow cytometers, fluorescence microscopes and other fluorescence

equipment, such as various microplate readers. The enzymatic product of (Z-DEVD)$_2$-Rh 110 has a much higher extinction coefficient, fluorescence quantum yield and photostability compared with the product of Z-DEVD-AFC. Additionally, (Z-DEVD)$_2$-Rh 110 is nonfluorescent, and generates highly fluorescent Rh 110 product upon cleavage of the DEVD blocking groups of (Z-DEVD)$_2$-Rh 110 by the caspase, while Z-DEVD-AFC itself still has strong residual fluorescence. In summary, these characteristics make (Z-DEVD)$_2$-Rh 110 a convenient and sensitive substrate for caspase-3. We have successfully used (Z-DEVD)$_2$-Rh 110 in flow cytometric analysis of apoptosis (data not shown). The flow cytometry analysis indicated that (Z-DEVD)$_2$-Rh 110 is at least 10 times more sensitive than Z-DEVD-AFC, which is consistent with our microplate assays, as described above.[8]

Acknowledgment: We thank Dr. Richard P. Haugland for his critical reading of the manuscript.

References and Notes

1. Keppler-Hafkemeyer, A.; Brinkmann, U.; Pastan, I. *Biochemistry* 1998, *37*, 16934.
2. Kumar, S. *Clin. Exp. Pharmacol. Physiol.* 1999, *26*, 295.
3. Porter, A. G.; Janicke, R. U. *Cell Death Differ.* 1999, *6*, 99.
4. Stennicke, H. R.; Salvesen, G. S. *J. Biol. Chem.* 1997, *272*, 25719.
5. (Z-DEVD)$_2$-Rh 110 was purified by HPLC using the following conditions: Prep LC2000 (Waters Corporation, Milford, MA); Prodigy Prep C18 reverse phase column (Phenomenex Corporation, Torrance, CA); linear gradient from 14% to 28% acetonitrile in 25 mM NH_4OAc (pH = 7); 65 mL/min flow rate. The structure was confirmed to elemental analysis, mass and NMR spectra.
6. Purified active recombinant human caspase-3 (Pharmingen, San Diego, CA) was used in the microplate assays.
7. Diwu, Z.; Lu, Y.; Zhang, C.; Klaubert, D. H. *Photochem. Photobiol.* 1997, *66*, 424.
8. Jurkat cells (American Type Culture Collection Co., Rockville, MD) were used in our flow cytometric assays. 10 μM camptothecin was used to induce apoptosis.